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Effects of Orally *Chlorella vulgaris* Algae Additive on Productive and Reproductive Performance of Lactating Friesian Cows

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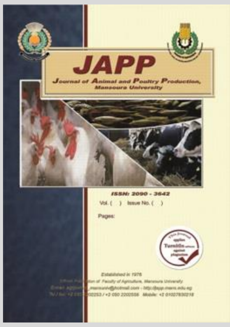

ABSTRACT

Fifteen multiparous Friesian cows with average live body weight (LBW) of 500±4.35 kg and 2-4 lactations were used after parturition and continues until 120 days of lactation and divided into three similar groups. Cows were fed a basal ration contained (DM basis) 40% concentrate feed mixture , 35% corn silage and 25% rice straw without any supplement in R1 (control) or orally supplemented with Chlorella algae and their media at the levels of 2 ml or 4 ml per kg LBW to instead of 1 and 2 liter/head/day for R2 and R3, respectively. Results showed that nutrients digestibility and feeding values improved significantly ($P<0.05$) with increasing the level of supplementation. Addition of C. vulgaris increased ($P< 0.05$) feed intake, the concentrations of total protein and globulin, AST to ALT ratio in serum, actual milk, 4% fat corrected milk yield, milk contents, also TVFA's compared with the control. However, the concentration of NH_3-N and Live enzymes activity decreased. Whereas, ruminal pH value and serum albumin and creatinine concentrations were nearly similar for the different groups. Chlorella supplementation had a significant improvements in feed conversion, economic efficiency and post-partum reproductive traits compared to control group. In conclusion, cows supplemented with Chlorella and their media at the level of 4 ml/kg LBW showed the best results concerning productive and reproductive traits and economic efficiency.

Keywords: Chlorella algae, productive and reproductive performance and economic efficiency

INTRODUCTION

The microalgae cultivating have been developed over the last decades because it is a simple and inexpensive method for CO_2 management, which is currently an important global issue. Otherwise, microalgae are capable of producing valuable metabolites, such as n-3 fatty acids for nutraceutical purposes (Guerin *et al.*, 2003; Hu, 2004). In recent years there has been increased interest in ways to manipulate the fatty acid composition of foods such as milk and milk products, because it contains a lot of health promoting components, such as n-3 fatty acids and conjugated linoleic acid (rumenic acid). These components could improve health of consumers. The high intake of n-3 PUFAs are able to reduce the risk factor of coronary heart disease, like the formation of blood clots leading to a heart attack (Li *et al.*, 2003).

Chlorella is a genus of single-celled green algae belonging to the division Chlorophyta. It is spherical in shape, about 2 to 10 µm in diameter, and is without flagella. Chlorella contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals (Scheffler, 2007). Chlorella kessleri is freshwater micro-algae specie, which contains high proportion of C18:3 (n-3) fatty acids. We hypothesized that milk fatty acid profile can be improved when the animals are fed with about 1% micro-algae supplemented diet and the same time serious negative effect on DM intake, milk composition and rumen function can be avoided (Póti *et al*., 2015).

Chlorella, a genus of unicellular green algae, is a good source of lutein. Compared with higher plants, chlorella has an advantage of being able to be cultivated in bioreactors on a large scale as a continuous and reliable source of product (Jeon *et al*., 2012). In the conditions of optimum growth, the biomass of Chlorella consists 25-50% of protein, 5-35% of carbohydrates and 5-20% of fat which is present as non-saturated fatty acids, the greater part of which is stearic, oleic, arachidonic, linolenic and linoleic acids, 5-10% of mineral substances, mostly consisting of phosphorus, sulfur and magnesium, and also carotene, vitamins C and K and vitamins of group B (Panahi *et al*., 2016). The microalgae contains peptides, alkaloids, polysaccharides, which can be used as both antimicrobial and antibacterial substances, and likewise, Chlorella has antioxidant properties, for it contains antioxidant enzymes, such as superoxide dismutase and catalase (Shibata *et al.*, 2003; Lee *et al*., 2010; Aliahmat *et al*., 2012; Zheng *et al*., 2012; Flerova and Bogdanova, 2014). Marine oil supplementation has also been displayed to have positive effects on reproduction with improved embryo quality and maintenance of pregnancies reported (Santos *et al*., 2008).

Additionally, C. vulgaris supplementation moderately increased milk yield, energy corrected milk, total solids, solids not fat and lactose. Feeding Algae diets increased milk unsaturated fatty acids with concomitant increases in total conjugated linoleic acid concentrations. The daily inclusion of 5 or 10 g of C. vulgaris in the diets of Damascus goats increased milk yield and positively modified milk fatty acid profile (Kholif *et al*., 2017).

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The aim of the present study was to investigate the effect of orally supplementation of Chlorella algae with their media on feed intake, digestibility, rumen fermentation, blood serum biochemical, milk yield and composition, feed conversion, economic efficiency and post - partum reproductive performance of Friesian cows.

MATERIALS AND METHODS

The present study was carried out at El-Karada Animal Production Research Station, Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation.

Chlorella vulgaris microalgae:

Lyophilized *Chlorella vulgaris* biomass was cultivated in Cyanobacteria Research Lab., Microbiology Dept., Sakha Agricultural Research Station, Kafr El-Sheikh, Soils, Water, and Environment Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Inoculum was prepared as described by El-Sayed *et al.* (2001) using BG-II growth medium (Stainer *et al*., 1971). Continuous light illumination was provided from daylight lamps (10×40 w). Aeration was achieved using an oil-free air compressor (HIBLOW AIR PUMP, type SPP-100GJ-H, Japan) through a 3-mm polyethylene tube. Room temperature was adjusted to 27±2 °C during the whole incubation period. Incubation was carried out using fully transparent polyethylene bags (75×5 cm2 and 100 µm thickness) containing 2.5 liters of algal broth. Mass production of C. vulgaris was performed within a 1000-litre Zigzag photobioreactor (El-Sayed *et al*., 2015). For harvesting and cleaning of the obtained biomass, a series of precipitation and washing was performed using tap water and a cooling centrifuge (RUNNE, HIDEBERG, RSV-20, Germany).

Animals and experimental groups

Fifteen Lactating Friesian cows with average live body weight (LBW) of 500±4.35 kg with mean metabolic body size $(BW)^{0.75}$ of 105 kg and 2-4 lactation seasons were used after parturition and continues until 120 days of lactation and divided into three similar groups (5 in each group). Cows were fed a basal ration contained (on DM basis) 40% concentrate feed mixture (CFM), 35% corn silage (CS) and 25% rice straw (RS) without any supplement in R1 which was served as control ration. The orally supplemented with Chlorella algae and their media at the levels of 2 ml (low level) or 4 ml (high level) / kg LBW to instead of 1 and 2 liter/head/day for R2 and R3, respectively. The Experimental ration was formulated to be 10.05 crude protein as recommended by (Qrskov , *et al* ., 1972). Chemical composition of feedstuffs and calculated composition of a basal ration are presented in Table (1).

Table 1. The Chemical composition of the feedstuffs and basal ration.

Item	DM	Chemical Composition (% as DM)					
	%	OM	CP	CF	EE	NFE	Ash
CFM	91.12	91.16	16.35	10.18	2.91	61.72	8.84
CS	30.65	93.73	8.42	26.70	2.54	56.07	6.27
RS	89.78	83.04	2.26	34.47	1.26	45.05	16.96
Basal ration	69.62	90.03	10.05	22.03	2.37	55.58	9.97

(CFM):concentrate feed mixture (CS): corn silage
(RS):rice straw Basal ration : 40% concentrate feed mixture, 35% corn silage and 25% rice straw

Concentrate feed mixture consisted of 17% undecorticated cotton seed cake, 20% undecorticated sunflower meal, 15% wheat bran, 32% yellow corn, 10% rice bran, 3% molasses, 2% limestone and 1% common sal

Management procedure

Cows were housed under sheds in semi-open backyards and were fed their rations to cover their recommended requirements according to NRC (2001). Concentrate feed mixture was offered in two equal parts daily at 8 a.m. and 4 p.m., corn silage and rice straw were offered once daily at 10 a.m. and 3 p.m., respectively. Chlorella with their media was added orally at morning every day before feeding. Animals were free for watering all the day round.

Digestibility trials

Digestibility trials were carried out at the end of the experimental period using 3 cows from each group to determine the digestibility coefficients and feeding values of the experimental rations using acid insoluble ash (AIA) as a natural marker (Van Keulen and Young, 1977). Feces samples were taken from the rectum of each cow twice daily at 12 h intervals during the collection period. Samples of feedstuffs were taken at the beginning, middle and end of the collection period. Representative samples of feedstuffs and faces were chemically analysed according to the methods of AOAC (2012). Digestibility coefficients were calculated from the equations stated by Schneider and Flatt (1975).

Rumen liquor samples

Rumen liquor samples were taken from animals at the same time of digestibility trial using stomach tube attached to a vacuum pump at 3 hours post feeding. Rumen liquor samples were strained through a double layer of cheese cloth and rumen pH was measured immediately after collection using a digital pH meter (Hanna Instruments pH). Rumen liquor was preserved with a few drops of saturated mercuric chloride and frozen in labelled poly propylene bottles for estimation of total volatile fatty acids (TVFA's) and ammonia nitrogen (NH_3-N). The TVFA's concentration was determined by a steam distillation method as described by Warner (1964) and NH3-N concentration was determined using magnesium oxide (AOAC, 2012).

Blood samples

Blood samples were taken from the jugular vein of each cow by clean sterile needle in a clean dry plastic tube after 3 hours from the morning feeding. Samples left in room temperature for 2 hours to coagulate and then centrifuged at 3000 rpm for 15 min to separate serum and stored at -20 oC. Total protein, albumin, globulin (total protein - albumin), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were determined calorimetrically by spectrophotometer (Spectronic 21D, USA) using commercial kits produced by Diagnostic System Laboratories, Inc., USA.

Milk yield and samples

Cows were mechanically milked at 6 a.m. and 5 p.m. Morning and evening milk yields were listed every day for each cow and also 4% FCM for each cow was calculated from daily milk yield and the percentage of fat in milk according to the formula of Gaines (1928): 4% FCM = Actual milk yield (kg) x 0.4 +15 x fat yield (kg). Milk samples were taken from each cow every two weeks from the consecutive evening and morning milkings and mixed in proportion to milk yield. Composite milk samples were analyzed for fat, protein, lactose, solids not fat (SNF), and total solids (TS) by Milko-Scan (model 133B), and ash by difference.

Feed conversion ratio and The Economic efficiency

Feed conversion ratio was determined as the amounts of DM, TDN and DCP required for producing 1 kg 4% FCM. The Economic efficiency was calculated as the ratio between the price of produced 4% FCM and the cost of feed consumed. The prices in Egyptian pound (LE) per ton were 4850 L.E. for concentrate feed mixture, 620 L.E. for corn silage and 650 L.E. for rice straw; 4 L.E./ liter Chlorella with their media and 5.50 L.E. / kg 4% FCM produced according to the prices of year 2018.

Reproductive parameters

Reproductive parameters as the periods from calving to first estrus and first insemination, days open, number of service per conception was recorded for every cows from the first service until conception and conception rate were listed for each cow.

Statistical analysis

The data were analyzed using general linear models (GLM) procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analyzed by Duncan's tests within SPSS program set at the level of significance P<0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

Nutrients digestibility and feeding values

Nutrients digestibility and feeding values for the experimental rations are shown in Table (2). The digestibility and feeding values improved significantly (P<0.05) with chlorella supplement as well as with the increase the level of supplementation. Ration 3 recorded significantly (P<0.05) the highest digestibility of DM, OM, CP and CF, followed by R2, whereas, R1 had the lowest values. Moreover, EE and NFE digestibility and also TDN and DCP values were significantly (P<0.05) higher for R3 than those of R1, while the corresponding values of R2 increased insignificantly (P>0.05) than those of R1. The determination of digestibility coefficients is the first step in evaluating the nutritional quality and utilization efficiency of an ingredient in complete diets for animals. These measurements provide an indication of the nutrients or energy fraction of the ingested feedstuffs that are not excreted in the feces, but are used for metabolic processes for animal production (NRC, 2011), thereby positively affecting production rates. Enhanced digestibility noted in C. vulgaris-supplemented cows may have resulted from improved ruminal fermentation kinetics. Chlorella vulgaris microalgae contains growth promoting substances such as S-nucleotide adenosyl peptide complex, which can affect digestibility (Han *et al*., 2002). Inclusion of C. vulgaris in the diets caused increased concentration of some bacterial species, e.g. Butyrivibrio fibrisolvens, Ruminococcus albus and Clostridium sticklandii, over forage-based diet in in vivo studies (Tsiplakou *et al.,* 2016), resulting in improved bacterial growth (Kotrbáček *et al.*, 2015). Futhermore, it contains β-glucan, which plays a role in scavenging free radicals (Iwamoto, 2004) and thus improves fermentation. Moreover, the C. vulgaris microalgae contents of PUFA(Polly unsaturated fatty acids), carotenoids, phycobiliproteins, polysaccharides and phycotoxins are required for higher microbial growth (Kotrbáček *et al.*, 2015). Tibbetts *et al.* (2016) notifed that the dietary effect of algal supplementation on feed digestibility in ruminants is related in part to its lipid content. Kholif *et al.* (2017) stated that *Chlorella vulgaris* supplementation to the diet of goats increased apparent diet digestibility compared with a control diet.

Table 2. Effect of feeding the experimental rations on nutrient digestibility coefficients and feeding values by cows.

Item	Experimental rations			SEM
	R1	R2	R3	
	Nutrient Digestibility (%)			
DM	63.38[c]	65.45[b]	67.73[a]	0.62
OM	64.45[c]	66.46[b]	68.66[a]	0.61
CP	64.49[c]	66.47[b]	68.64[a]	0.58
CF	61.60[c]	63.78[b]	66.17[a]	0.64
EE	72.27[b]	73.83[ab]	75.53[a]	0.53
NFE	68.04[b]	69.78[ab]	71.70[a]	0.56
	Feeding values %			
TDN	61.72[b]	63.45[ab]	65.36[a]	0.54
DCP	6.48[b]	6.68[ab]	6.90[a]	0.06

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).

Feed intake

Feed intake by lactating cows with mean metabolic body size $(BW)^{0.75}$ of 105 kg . fed the different rations is presented in Table (3).

Table 3. Average daily feed intake (kg DM/ h/day) and chlorella (liter/day) for different experimental rations. (kg DM/h/day)

Item	Experimental rations			SEM
	R1	R2	R3	
L.B.W	500	498	502	2.05
CFM	6.24[c]	6.39[b]	6.53[a]	0.05
CS	5.46[c]	5.58[b]	5.72[a]	0.04
RS	3.9[c]	3.99[b]	4.09[a]	0.03
Total DM	15.60[c]	15.96[b]	16.34[a]	0.11
TDN	9.62[c]	10.13[b]	10.68[a]	0.14
CP	1.57[c]	1.60[b]	1.64[a]	0.01
DCP	1.01[c]	1.07[b]	1.13[a]	0.02
Chlorella	0[c]	1[b]	2[a]	0.25

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).

The C. vulgaris supplementation increased (P< 0.05) feed intake compared with the control diet as well as with increasing the level of additive. Ration 3 revealed significantly (P<0.05) the highest intake of concentrate feed mixture, corn silage, rice straw, DM, TDN, CP and DCP followed by R2, while the lowest amounts of intake was detected in control one (R1). In spite of increasing DM and CP intake by 2.31 and 1.91%, while the intake of TDN and DCP increased by 5.30 and 5.94% for R2 (low chlorella level) compared to R1, respectively. The corresponding values for high chlorella level (R3), DM and CP intake by 4.74 and 4.46%, while the intake of TDN and DCP increased by 11.02 and 11.88% compared to R1, respectively. These increases might be attributed to the increase of TDN and DCP values with chlorella supplementation (Table 2). Enhanced digestibility will lead to increased rate of passage, resulting in greater DMI (Tibbetts *et al.*, 2016). Kholif *et al.* (2017) reported that *Chlorella vulgaris* treatments increased feed intake.

However, Glover *et al.* (2012) observed that supplementing diets of lactating cows with marine microalgae at 200 g/day did not affect DMI of fresh forage or silage-based total mixed ration. Inconsistency between these studies may be due to different diet composition and different algae inclusion doses (Reynolds *et al.*, 2006).

Rumen fermentation activity

Results of rumen fermentation activity in Table (4) revealed that Chlorella vulgaris had no significant effect on ruminal pH values. There was a slight increase with increasing chlorella level being 6.62, 6.66 and 6.68 for R1, R2 and R3, respectively. These results agreed with the finding of Van Soest (1983) stated that the optimum pH value for growth of cellulytic microorganisms was 6.7 and the range for normal condition was about ±0.5 pH degree. While, the concentration of NH_3-N decreased and TVFA's increased significantly (P<0.05) with chlorella as well as with the level of supplementation. Availability of peptides and amino acids are stimulatory factors for microbial growth and ruminal digestion (Carro and Miller 1999), particularly in the presence of rapidly fermentable carbohydrate. Chakraborty *et al.* (2013) reported that glucose represents the main sugar of Chlorella microalgae, with about 630–900 g/kg of total sugars as glucose. Increased proportion of propionic acid is considered beneficial in ruminant nutrition and dairy production, as propionate is the primary gluconeogenic VFA that can be used for lactose biosynthesis (Vanhatalo *et al*., 2003). Kholif *et al*. (2017) notified that *Chlorella vulgaris* supplementation to the diet of goats increased ruminal TVFA's concentration compared with a control diet.

Table 4. Effect of feeding experimental rations on some rumen liquor parameters and blood serum biochemical at 3 hr after feeding.

Item	Experimental rations R1	R2	R3	SEM
Rumen fermentation activity				
pH value	6.62	6.66	6.68	0.05
NH3-N (mg/100 ml)	19.23[a]	17.47[b]	15.55[c]	0.46
TVFA's (meq/100 ml)	16.68[c]	19.26[b]	20.82[a]	0.52
Blood serum biochemical				
Total protein (g/dl)	7.16[b]	7.55[ab]	7.80[a]	0.12
Albumin (g/dl)	3.64	3.54	3.55	0.03
Globulin (g/dl)	3.52[b]	4.02[ab]	4.24[a]	0.14
Albumin: globulin ratio	1.05[a]	0.89[ab]	0.84[b]	0.04
AST (IU/L)	42.59[a]	39.77[ab]	37.07[b]	1.04
ALT (IU/L)	17.37[a]	15.25[ab]	13.00[b]	0.67
AST / ALT	2.45[c]	2.61[b]	2.85[a]	0.08
Creatinine (mg/dl)	0.78	0.73	0.71	0.01

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).

Blood serum biochemical

Blood serum biochemical of cows in the different groups are presented in Table (4). Results showed significant differences (P<0.05) in total protein, globulin, albumin to globulin ratio, AST and ALT among the tested rations. The concentrations of the total protein and globulin in serum of cows supplemented with high chlorella level (R3) were significantly (P<0.05) higher than those of low level (R2) and the control, whereas values of low chlorella level (R2) were insignificant increased than the control (R1). While, serum albumin concentration was nearly similar for the different groups. Albumin to globulin ratio was significantly (P<0.05) higher in R1 (1.05) compared to the other groups R2 and R3 (0.89 and 0.84) respectively. Albumin to globulin ratio in groups received chlorella improved cows health, which were within the normal range for good health being 0.80 to 0.95. Live enzymes activity (AST and ALT) decreased markedly (P<0.05) with *Chlorella* supplementation in R2 and R3. While AST to ALT ratio was significantly (P<0.05) higher in R3 (2.85) compared to the other groups R2 and R1 (2.61 and 2.45) respectively while the same time, serum creatinine concentration tended to decrease with chlorella supplementation. Increased serum total protein with *C. vulgaris* supplementation may be due to increased DCP intake. Reduced concentrations of AST and ALT are considered as important indicators for liver activity, functionality and hepatotoxicity, suggesting the absence of pathological lesions in the liver. In the present study, feeding *C. vulgaris* microalgae, at both levels, resulted in a significant decrease in AST and ALT concentrations, indicating a probable protective role for *C. vulgaris* against liver dysfunction. Kholif *et al.* (2017) reported that *Chlorella vulgaris* treatments increased serum total protein concentration but decreased serum ALT and AST concentrations. Also, El-Abd and Hamouda (2017) found that watering chickens with algae caused a reduction in creatinine, AST and ALT.

Milk yield and composition

Milk yield of cows fed the different rations are presented in Table (5). Actual milk and 4% fat corrected milk (FCM) yield of cows fed the high level of chlorella (R3) were significantly (P<0.05) higher than those of cows fed low level of chlorella (R2) or control ration (R1).

Table 5. Milk yield and composition for different experimental rations.

Item	Experimental rations R1	R2	R3	SEM
Milk yield (kg/day)				
Actual milk	13.58[c]	15.20[b]	16.81[a]	0.42
4% FCM	12.81[c]	14.62[b]	16.47[a]	0.47
Milk composition (%)				
Fat	3.62[b]	3.74[ab]	3.87[a]	0.04
Protein	2.66[b]	2.76[ab]	2.90[a]	0.04
Lactose	4.67[b]	4.80[ab]	4.90[a]	0.04
SNF	8.03[c]	8.26[b]	8.51[a]	0.07
TS	11.66[c]	12.01[b]	12.38[a]	0.10
Ash	0.71	0.71	0.71	0.02
Density	23.45[b]	23.98[ab]	24.60[a]	0.23
Milk composition (g/kg)				
Fat	36.2[b]	37.4[ab]	38.7[a]	0.4
Protein	26.6[b]	27.6[ab]	29.0[a]	0.4
Lactose	46.7[b]	48.0[ab]	49.0[a]	0.4
SNF	80.3[c]	82.6[b]	85.1[a]	0.7
TS	116.6[c]	120.1[b]	123.8[a]	1.0
Ash	7.1	7.1	7.1	0.02
Milk Constituents (g/day)				
Fat	491.596[c]	568.48[b]	650.547[a]	0.17
Protein	361.228[c]	419.52[b]	487.49[a]	0.17
Lactose	634.186[c]	729.6[b]	823.69[a]	0.17
SNF	1090.474[c]	1255.52[b]	1430.53[a]	0.29
TS	1583.428[c]	1825.52[b]	2081.078[a]	0.42
Ash	96.418[c]	107.92[b]	119.351[a]	0.08

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).

Furthermore, the yield of actual and 4% FCM yield of cows fed R2 were significantly higher than (P<0.05) the R1. Actual milk yield of R2 and R3 were increased by 1.62 and 3.23 kg/day or 11.93 and 23.78% compared to R1, respectively. The corresponding values of 4% FCM were 1.81 and 3.66 kg/day or 14.13 and 28.57%, respectively.

Increased intake and digestibility observed in cows fed C. vulgaris may have resulted in higher milk production.

Moreover, C. vulgaris contains several nutrients, such as amino acids, essential fatty acids, vitamins and minerals that could enhance milk production (Lum *et al*., 2013). In addition, propionate is the precursor for gluconeogenesis and lactose synthesis, and increasing glucogenic precursors will have a favourable effect on milk yield (Rigout *et al*., 2003). Higher amounts of milk (10.3 and 12.4%, respectively) and FCM (11.1 and 13.2%, respectively) were noted in goats fed 5 and 10 Chlorella diets (Kholif *et al*., 2017). Daily yield of actual milk and 4% FCM were increased significantly (P<0.05) with Spirulina addition, where R3 (high Spirulina level) listed significantly (P<0.05) the highest daily actual milk and 4% FCM yield followed by R2 (low Spirulina level), while R1(control ration) had the lowest yield (Gaafar *et al*., 2017).

Milk composition

Concerning milk composition in Table (5) showed that the contents of fat, protein and lactose in milk as well as density of milk were increased significantly (P<0.05) for R3 than the R1, but without significant effect between R1 and R2 . While, the contents of SNF and TS were significantly higher (P<0.05) for R3 than that of R1 and R2 and it was significantly (P<0.05) higher for R2 than that of R1. The yield of milk constituents reflect the variations in milk yield and milk composition among the different groups. These results agreed with the findings of Kholif *et al.* (2017) reported that milk contents from total solids (P=0·010), solids not fat (P<0·001) and lactose (P=0·001) were higher in Alg05 and Alg10-fed goats. Papadopoulos *et al.* (2002) found that milk fat content was significantly increased (P < 0.001) for treatment high algae level, whereas milk protein content was significantly increased (P < 0.001) for all levels of algae treatments. Gaafar *et al.* (2017) stated that the high level of Spirulina (2 ml/kg LBW) showed significantly (P<0.05) the highest concentrations of fat, protein, lactose, solids not fat (SNF) and total solids (TS) followed by low level (1 ml/kg LBW), while G1 had the lowest values. Simkus *et al.* (2008) who showed an increase in milk fat (between 17.6% and 25.0%), milk protein (up by 9.7%) and lactose (up by 11.7%) in cows receiving Spirulina compared to those free from Spirulina supplement.

Feed conversion and economic efficiency

Feed conversion ratio expressed as the amounts of DM, TDN, CP and DCP per 1 kg 4% FCM as affected by Chlorella additive are shown in Table (6). Chlorella algae additive led to a significant decrease (P<0.05) in the amounts of DM, TDN, CP and DCP per 1 kg 4% FCM, which R3 recorded the lowest values followed by R2, while R1 had the highest values. The amounts of feed/kg 4% FCM for R2 and R3 decreased by 10.66 and 18.85% for DM, 8.00 and 13.33% for TDN, 10.17 and 18.55% for CP and 7.58 and 13.32% for DCP compared to R1, respectively. The improvements of feed conversion ratio with Chlorella may be attributed to the improvements in nutrients digestibility (Table 2), feed intake (Table 3), rumen fermentation activity (Table 4) and milk yield (Table 5). These results are in accordance with those obtained by El-Sabagh *et al.* (2014) found that Spirulina supplementation in fattening lamb's diets improved feed conversion ratio, compared to the control group (P<0.05). Also, Mariey *et al*. (2014) reported that the birds which fed Spirulina diets achieved superior means of feed conversion ratio compared to those of the control group. Kholif *et al*. (2017) stated that milk yield and energy corrected milk yield per kg DM intake improved with Chlorella additive at levels of 5 and 10 g for dairy goats. Gaafar *et al*. (2017) showed that feed conversion improved with Spirulina additive as well as with increasing the level of additive in ration of dairy cows.

Table 6. Feed conversion and economic efficiency for different experimental rations.

Item	Experimental rations			SEM
	R1	R2	R3	
Feed conversion				
DM (kg/kg 4% FCM)	1.22[a]	1.09[b]	0.99[c]	0.03
TDN (kg/kg 4% FCM)	0.75[a]	0.69[b]	0.65[c]	0.01
CP (g/kg 4% FCM)	122.44[a]	109.81[b]	99.73[c]	2.87
DCP (g/kg 4% FCM)	78.98[a]	72.99[b]	68.46[c]	1.43
Economic efficiency				
Feed cost (LE/day)	47.07[c]	52.18[b]	57.31[a]	1.27
Feed cost (LE/kg 4% FCM)	3.68[a]	3.57[ab]	3.48[b]	0.03
Output of 4% FCM (LE/day)	70.46[c]	80.40[b]	90.60[a]	2.58
Net revenue (LE/day)	23.39[c]	28.21[b]	33.29[a]	1.34
Net revenue improvement %	00.00[c]	21.00[b]	42.91[a]	5.75
Economic efficiency[1]	1.50[b]	1.54[ab]	1.58[a]	0.01
Economic efficiency[2] %	49.65[b]	54.04[ab]	58.06[a]	1.35

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).
[1] Economic efficiency = output of 4% FCM/ feed cost.
[2] Economic efficiency = net revenue * 100/ feed cost.

Data of economic efficiency was presented in Table (6) data showed that Chlorella additive resulted in significant (P<0.05) improvements in economic efficiency. Daily feed cost, total output of milk yield, daily net revenue and net revenue improvement increased significantly (P<0.05), becouse, feed cost per 1 kg 4% FCM was decreased significantly (P<0.05) with Chlorella compared to the control. High Chlorella level (R3) listed significantly (P<0.05) the higher feed cost, total output of milk yield, net revenue and net revenue improvement compared to low level (R2) and control one. While, it was significantly higher (P<0.05) for low level (R2) than that of R1 (control). Futhermore, economic efficiency as the ratio between total output and feed cost or as the percentage of net revenue to feed cost were significantly higher (P<0.05) with high chlorella level than that of control one. Net revenue increased by 21.00 and 42.91% for R2 and R3 compared to R1, respectively. These results agreed with those obtained by Kulpys *et al*. (2009) found that throughout the 90day experiment, the average income from the milk of one cow from the experimental group was 378 LE or 21% more than that of controlled group. The use of cyanobacteria additives was economically effective because 1 LE costs for Spirulina platensis increased income from milk by 8.4 LE. Gaafar *et al.* (2017) stated that in spite of Spirulina additive for dairy cows increased feed cost, whereas increased the total output of milk yield, net revenue and economic efficiency.

Reproductive performance

Post-partum reproductive parameters of Friesian cows as affected by Chlorella additive are shown in Table (7). Chlorella improved significantly (P<0.05) all the postpartum reproductive parameters. Cows in R3 recorded significantly (P<0.05) the short periods from parturition until the first estrus and insemination, as well as days open followed by R2, while those in R1 had the longer periods. Moreover, cows in R3 showed significantly (P<0.05) the highest conception rate and the lowest number of service per conception followed by R2, while those in R1 revealed the opposite trend. These results agreed with those obtained by Gaafar *et al* . (2017) found that cows supplemented with Spirulina recorded significantly (P<0.05) the short periods from parturition until the first estrus and insemination, as well as days open, the highest conception rate and the lowest number of service per conception.

Table 7. Post-partum reproductive performance for different experimental rations.

Item	Experimental rations			SEM
	R1	R2	R3	
First estrus (day)	49.75[a]	39.64[b]	31.18[c]	1.96
First insemination (day)	86.92[a]	66.70[b]	51.46[c]	3.82
Days open (day)	125.18[a]	94.80[b]	72.35[c]	5.67
No. service/conception	2.30[a]	1.65[b]	1.15[c]	0.15
Conception rate %	60.00[c]	80.00[b]	100.00[a]	9.56

a, b, c: Means in the row with different superscripts differ significantly (P<0.05).

CONCLUSION

Friesian cows which supplemented with orally Chlorella and their media at the level of 4 ml / kg LBW gave the best results concerning nutrients digestibility, feed intake, rumen fermentation activity, blood biochemical parameters, milk yield and composition, feed conversion, economic efficiency and post-partum reproductive traits**.**

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تأثير إضافة طحلب الكلوريلا على الأداء الإنتاجى والتناسلى للأبقار الفريزيان الحلابة

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أجريت هذه الدراسة على ١٥ بقرة فريزيان متعددة الولادات متوسط الوزن الحى ٥٠٠±٤,٣٥ كجم ومواسم الحليب من ٢-٤، بعد الولادة واستمرت لمدة ١٢٠ يوم من موسم الحليب. قسمت الأبقار الى ثلاثة مجموعات متماثلة . غذيت الأبقار على العليقة الأساسية التى تتكون من ٤٠% علف مركز ، ٣٥% سيلاج أذرة ، ٢٥% قش أرز. المجموعة الأولى بدون إضافة والتى اعتبرت مجموعة المقارنة، بينما تم إضافة طحلب الكلوريلا والبيئة النامى عليها بتجريع الحيوانات بمعدل ٢ مل ، ٤ مل لكل واحد كجم وزن حى أى بمعدل ١ لتر ، ٢ لتر فى المجموعة الثانية والثالثة على التوالى .أوضحت النتائج تحسن معنوى فى معاملات الهضم والقيم الغذائية للعلائق مع زادة مستوى إضافة طحلب الكلوريلا ، كما أن إضافة طحلب الكلوريلا أدت الى زيادة معنويةً فى كمية الغذاء المأكول ، ارتفاع تركيز البروتينات الكلية والجلوبيولين فى سيرم الدم ، نسبة AST إلى ALT ، إنتاج اللبن الفعلى والمعدل الدهن ٤% ومكونات اللبن وكذلك الأحماض الدهنية الطيارة بالكرش مقارنة بالكنترول فى حين أن إضافة الطحلب ليس لها أى تأثير معنوى على قيم الأس الهيدروجينى للكرش بينما انخفضت أمونيا الكرش وكذلك نشاط إنزيمى الكبد ، فى نفس الوقت تشابهت تقريباً تركيزات الألبيومين والكرياتنين فى الدم ، أوضحت النتائج أن زيادة مستوى الإضافة طحلب الكلوريلا أدت إلى انخفاض معنوى فى كميات المادة الجافة والمركبات الكلية المهضومة والبروتين الخام والبروتين المهضوم اللا زمة لانتاج ١ كجم لبن معدل الدهن ٤% مقارنة بمجموعة المقارنة ، وأظهرت أيضاً تحسن معنوى للصفات التناسلية وكذلك تحسن معنوى للكفاءة الإقتصادية نستخلص من هذه الدراسة أن اضافة طحلب الكلوريلا والبيئة النامية عليها بمعدل ٤ مل/ كجم وزن حى بالتجريع للأبقار الفريزيان حققت أفضل آداء إنتاجى ، بعض الصفات التناسلية بعد الولادة وكذلك الكفاءة الاقتصادية.